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Peroxisome Proliferator-Activated Receptor (PPAR): Structure, Mechanisms of Activation and Diverse Functions

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Key words: peroxisome/peroxisome proliferator/PPAR/orphan receptor/hepatocarcinogenesis

ABSTRACT. **The structurally diverse xenobiotic peroxisome proliferators (PPs) increase the number of peroxisomes per cell and the levels of several enzymes, and cause hepatomegaly, often leading to hepatocarcinogenesis in a species- and tissue-specific manner. The deadlocked problems of the molecular mechanism of PP action and its physiological meanings have begun to be understood through cDNA cloning of a PP-activated receptor (PPAR). PPAR, a member of the steroid/thyroid/vitamin superfamily of nuclear receptors, has isoforms and differentially heterodimerizes with other nuclear receptors, providing potential mechanisms not only for species- and tissue-specific actions but also for diverse actions of PPs. Recent findings related to PPAR are summarized, and its possible role in lipid metabolism and involvement in PP-induced hepatocarcinogenesis are discussed.**

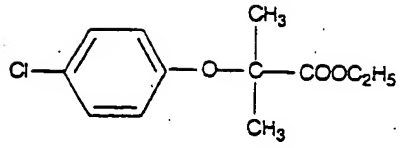
Peroxisomes are organelles that are involved in diverse functions, including the β-oxidation of fatty acids (see Refs. 48, 51 for reviews). They are found in most eukaryotic cells and their essential role has been emphasized by the discoveries of several human disorders caused by the lack of peroxisomes (see Ref. 49 for review). In addition to containing H_2O_2-producing oxidases (12), peroxisomes are unique for their ability to proliferate in response to several structurally disparate chemicals, which are designated "peroxisome proliferators (PPs)", in rodent liver cells (51, 77) (see Fig. 1). One class of proliferators is certain hypolipidemic drugs, such as clofibrate and its analogs (ciprofibrate, bezafibrate, and nafenopin) (59, 77). Some hypolipidemic drugs having no obvious structural similarity to clofibrate, such as Wy-14,643 and tibric acid, are also potent proliferators (76), as are certain phthalate-ester plasticizers such as DEHP (41).

All the structurally diverse PPs are thought to induce peroxisomal gene transcription through the same mechanism (75). A receptor-mediated mechanism hypothesis was presented and biochemical approaches were made to detect the PP receptor. Although a specific binding for PPs (43, 44, but also see Ref. 58) and purification of the binding protein (2) were reported, further characterization which would facilitate our understanding of the mechanism of the PP action has been unsuccessful. The recent isolation and characterization of the PP-activated receptor (PPAR) cDNA (30), which was unexpected by most researchers in this field, was a breakthrough in our knowledge of the molecular mechanism of the transcriptional activation by PPs and the endogenous significance of the regulation by the receptor. This review highlights these findings and discusses the possible diverse actions of PPs with emphasis on their role(s) in PP-induced hepatocarcinogenesis, because another important aspect of PPs is that they cause hepatomegaly and several of them are non-genotoxic but have hepatocarcinogenic properties (see Refs. 60, 74 for reviews).

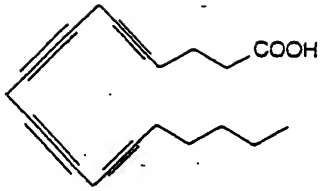
Isolation of PPAR cDNA.

In 1990, Issemann and Green (30) reported cDNA cloning of a new member in the steroid/thyroid/vitamin superfamily of nuclear receptors. With neither information on the actual ligand for the receptor nor the binding DNA element, they hypothesized a steroid hormone-like mechanism for the PP action and isolated three new receptor cDNA clones from a mouse cDNA library using only the information on the amino acid conservation in the DNA-binding domain of the superfamily members (15, 47). One of them was called PPAR (PP-activated receptor) because the chimera receptor constructs encompassing the N-terminal, the *trans*-acti-

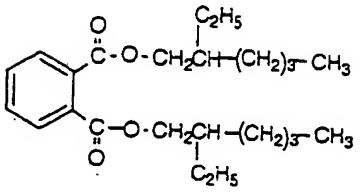
Abbreviations used: PP, peroxisome proliferator; PPAR, PP-activated receptor; Wy-14,643, [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid; DEHP, di(2-ethylhexyl)phthalate; PPRE, PP response element; DR-n, direct repeat separated by n base pairs; RXR, retinoid X receptor; RAR, retinoic acid receptor; VDR, vitamin D receptor; TR, thyroid receptor; TRE, thyroid hormone response element; RXRE, retinoid X response element; ARP-1, apoAI regulatory protein-1; HNF, hepatocyte nuclear factor.



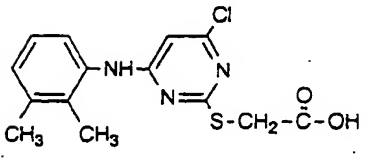
Clofibrate



5,8,11,14-Eicosatetraynoic acid (ETYA)



DEHP : di(2-ethylhexyl)phthalate



Wy-14.643 : [4-chloro-6-(2.3-xylidino)-2-pyrimidinylthio] acetic acid

Fig. 1. Structures of typical PPs mentioned in this review.

vating and the central DNA-binding domains of the glucocorticoid or estrogen receptor linked to the C-terminal, the putative ligand-binding domain of the PPAR, were activated by a structurally diverse group of PPs with the same efficiency as this group induces peroxisomal β-oxidation enzymes in the rodent hepatocytes (30). Similar forcible approaches were successful for the isolation of the cognate receptors from other species and some previously isolated orphan receptors that were putative receptors for functions and ligands to be identified were uncovered as PPARs by homology with the mouse sequence and functional analyses (see below). The frequency of isolation of PPARs from various tissues of different species seems high in spite of the estimation that the number of orphan receptors exceeds 50 (68), suggesting their abundance and involvement in fundamental cellular processes.

Structure of PPAR and evolution of the gene.

Subsequent to the pioneering work on the cloning of mouse PPAR, independent clonings of three PPARs of *Xenopus laevis* (14), rat (21), and human (80) were reported. Another human PPAR was also cloned by cross hybridization using the mouse probe (83). Because of the cloning strategies employed, it is natural that all PPARs belong to a superfamily of transcriptional regulatory factors which include steroid hormone, thyroid hormone, vitamin D3 and retinoid receptors (for a review see 47). These receptors have a modular structure consisting of six functional domains, defined as A, B, C, D, E and F (42). Region C containing about 66 amino acids forms two zinc fingers and functions as the core of the DNA-binding domain (47). The amino acid sequences in region C of PPARs so far reported are compared in Fig. 2. The number of amino acids between the first two of the four conserved Cys residues in the second zinc-binding site of all PPARs is three instead of five as found in all other nearly 40 (47) members of this family except the *tailless* orphan receptor which has seven residues (71). The sequence homologies in the domains of PPARs are high enough to distinguish them from other members of the superfamily, but the differences among PPAR isoforms are not as evident. Isoform classification of mammalian PPARs relative to *Xenopus* α, β and γ receptor (14) is only tentative at present and several other members in this subfamily may be cloned even in *Xenopus*. Further detailed structural and functional analyses including other domains are also necessary.

Dreyer *et al.* (13) have made an evolutional analysis by comparing the central conserved portions (implicated in the regulation of transactivation and dimerization, Ti-DM) of the E regions (the ligand-binding domains) of PPARs with those of other nuclear hormone receptors. According to their phylogenetic tree (Fig. 3), the appearance of the PPAR group corresponds to that of the early vertebrates and the divergence of the three receptor genes found in *Xenopus laevis* (14) predates the dichotomy between mouse and *Xenopus* PPARα. Their analysis strongly suggests that mammalian species

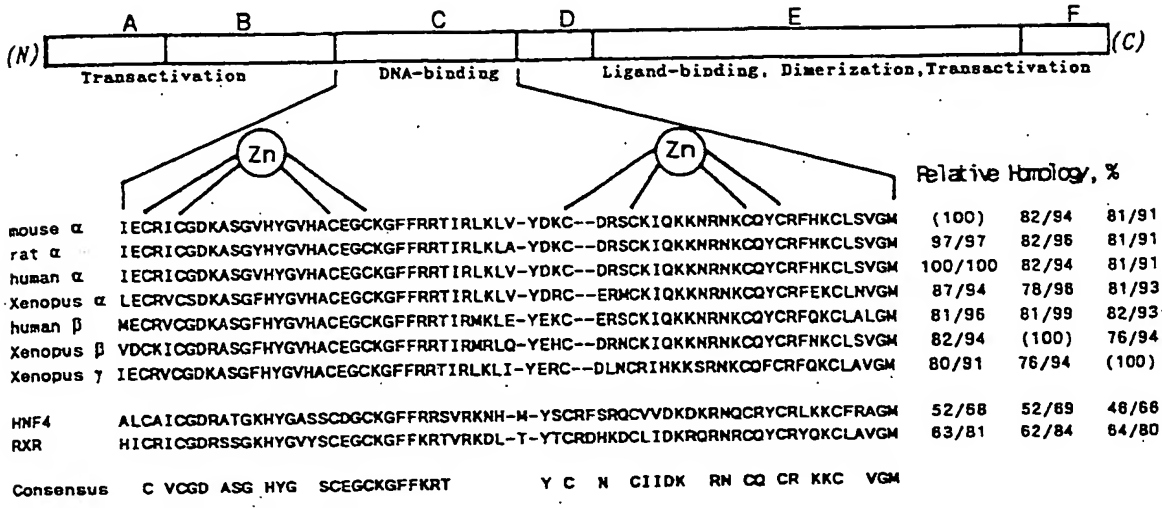


Fig. 2. Organization of different functional domains in members of the steroid/thyroid/vitamin superfamily and sequence comparison of the DNA binding (C) domains in PPARs. Those of rat HNF4 and human RXR which are discussed in the text are also compared along with the consensus sequence deduced from all members of the superfamily (Ref. 47). Sequences were aligned to maximize the homologies by introducing sequence gaps. Sequence homologies relative to mouse α, Xenopus β, or Xenopus γ RRAR sequence were calculated using the numbers of identical (numerators) or homologous (denominators) amino acids. Those amino acids enclosed in the same set of parentheses are regarded as homologous: (Ala, Ser, Thr, Pro, Gly); (Asn, Asp, Glu, Gln); (His, Arg, Lys); (Met, Leu, Ile, Val); (Phe, Tyr, Trp).

also have a PPAR subfamily consisting of several receptor isoforms. In human, two different cDNA clones have been obtained by independent approaches (80, 83). Several protein bands besides an expected 53 kDa PPARα were detected in an extract from PP-treated rat liver by immunoblotting using polyclonal antibodies against a portion of recombinant mouse PPARα which is the least conserved region among nuclear hormone receptors (19). This may suggest that PPAR isoforms also exist in rodent species, although further characterization of their identities is necessary.

DNA binding of PPAR.

PPAR is thought to have two binding sites: one for a specific DNA element and another for an *in vivo* ligand, but neither was known at the time of cDNA cloning. Characterization of the DNA binding came first. Involvement of PPAR in transcriptional activation of the genes in the peroxisomal β-oxidation pathway by PPs has been supported by identification of PP response element (PPRE) in the genes and by demonstration of the receptor binding to the element (87). Although the DNA motif TGACC has been expected as a part of the sequence recognized by PPAR from the deduced amino acid sequence of the first zinc finger region of the mouse receptor, the pioneering and frontal work of Osumi *et al.* (69) on the promoter structure of the rat acyl CoA oxidase gene played an essential role in the identification of the PPRE. They have already identified the positive enhancer (−578/−553) containing TGACCTTTGTCCT in the promoter by the conventional deletion-transfection method. Largely based on the result of the rat gene, Tugwood *et al.* (87) finaly identified PPRE as an almost perfect direct repeat of the sequence TGA/TCCT separated by one base pair (DR-1). This element has been found not only in the rodent acyl CoA oxidase genes but also in other PP inducible genes such as 3-ketoacyl-CoA thiolase (27), enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (54, 96, 98), cytochrome P450IV family (5, 65), fatty acid binding protein (6, 31, 91) and malic enzyme (26).

Interestingly, PPRE is indistinguishable from the previously identified retinoid X response element (RXRE) (53) (see Fig. 4). RXR has already been characterized to form heterodimers with VDR (vitamin D), TR (thyroid hormone) and RAR (retinoic acid), and to bind cooperatively to their cognate response elements (38, 39, 88). It was therefore plausible to test the possibility that PPAR can be a counterpart of an RXR heterodimer. Thus, mouse RXRα has been shown to be almost essential for the binding of PPARα to PPRE, exerting ligand-dependent synergistic activation of the PPRE-containing promoters (3, 20, 32, 40, 97) (see Fig. 4). Heterodimer formation and synergistic interaction between *Xenopus* PPARα and mouse RXRβ (35) were also demonstrated. *In vitro* binding and *in vivo* co-transfection studies showed that the PPARα-RXRα complex has a preference for the direct repeat separated by 1 bp. PPAR may

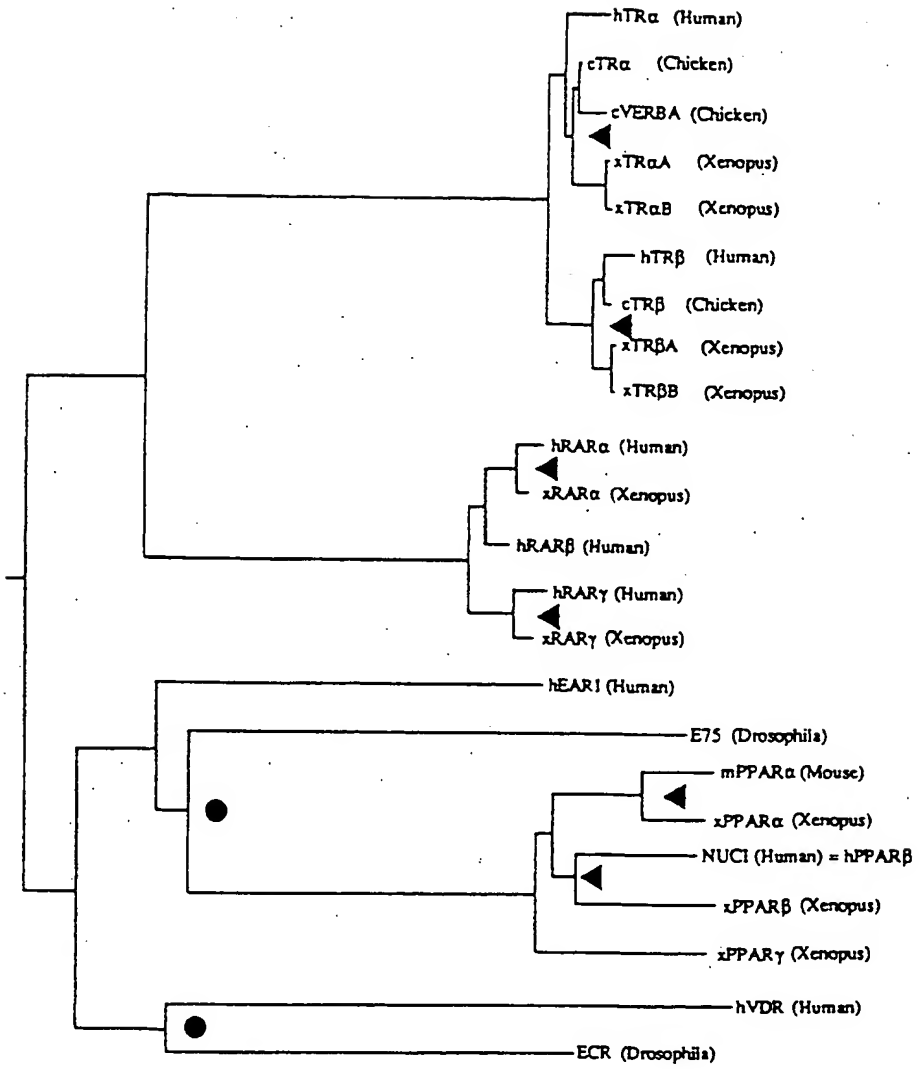


Fig. 3. Phylogenetic tree connecting the members of the first subfamily of nuclear hormone receptors based on sequence comparisons of the central conserved portion of the ligand-binding domains (Ti-DM). An arrow indicates the dichotomy between arthropod and vertebrate and asterisks point out those between mammalian and Xenopus genes. Modified from Dreyer *et al.* (13).

form heterodimers with other nuclear receptors, and differential heterodimerization of PPAR with various receptors provides a potential mechanism for not only specific but also diverse actions of PPs as discussed below.

It should be noted, however, that PPAR-RXR heterodimerization has not been demonstrated in the cell containing normal levels of these factors. In this context, the result of Osumi *et al.* (69), showing that a few adjacent nucleotides downstream to the PPRE of the rat acyl CoA oxidase gene were essential for the PP-mediated transcriptional activation in hepatoma cells transfected only with the target gene, is interesting.

In vivo ligand for PPAR.

Although the PPARs have been shown to be activated by structurally diverse PPs, no direct evidence to indicate the interaction between the receptor and a PP has been reported. (A preliminary result showing that recombinant PPAR specifically bound medium- and long-chain fatty acids *in vitro* was reported at "the FEBS Satellite International Meeting on Cellular Aspects Related to Peroxisomes" held in Dijon on April 28–29, 1993.) Structurally unrelated PPs may not specifically bind to the same site of the same PPAR, but the molecules of similar structures, whether they came

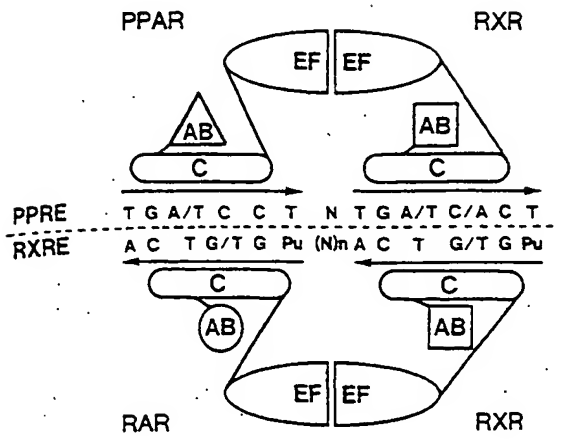


Fig. 4. Schematic diagram showing heterodimer formations of PPAR-RXR on PPRE and PAR-RXR on RXRE. PPRE, TGA/TCCT (DR-1) and RXRE, PuGG/TTCA (DR-1, in this case) are shown complementary to each other to emphasize their similarities. Relative positions of the receptors that recognize direct repeats can be exchanged on the element, but the heterodimers will form stereoscopically different complexes from the previous ones because of asymmetry of the element (see Ref. 50).

from outside the cell and remained inside as a result of poor metabolism or increased inside the cell due to intracellular metabolism or the effects of PPs on the metabolism, are plausibly the ligands for PPs. Unmetabolizable proliferators such as perfluorinated fatty acids (1, 29), for example, will perturb the lipid metabolism and increase the level of *in vivo* ligands, thus activating the PPAR.

Göttlichter *et al.* (21) examined the possibility that some intermediates in lipid metabolism may be physiological activators of PPAR using an easily detectable chimeric PPAR expressed in CHO cells. Among the compounds related to lipid metabolism, the physiological concentrations of fatty acids with the chain lengths of $n>6$, like linoleic (C18 : 2), arachidonic (C20 : 4), or lauric acid, but not cholesterol, 25-hydroxycholesterol, or dehydroepiandrosterone, activate the chimeric receptor depending on the putative ligand-binding domain of rat PPAR. Dreyer *et al.* (13, 14) employed a similar approach using a *Xenopus* PPAR and reached essentially the same conclusion with a finding that 5,8,11,14-eicosatetraynoic acid, an alkyne homologue of arachidonic acid and a competitive inhibitor of the lipoxygenase and cyclooxygenase, was an activator 100 times more effieient than the previously most potent Wy-14,643 (13, 36). Banner *et al.* (2a) recently identified free fatty acids as PPAR activators in human plasma by the method combining physicochemical fractionation and biological assay using CHO cells stably expressing the chimeric receptor.

Activation of PPAR by fatty acids to regulate expression of the genes for the peroxisomal β-oxidation system and fatty acid metabolizing P450IV family suggests the possibility that PPAR plays a physiological and essential role in the autoregulatory loop in lipid homeostasis (21, 72). The presence of isoforms of PPAR may be related to the smooth regulation of the system. Differ-

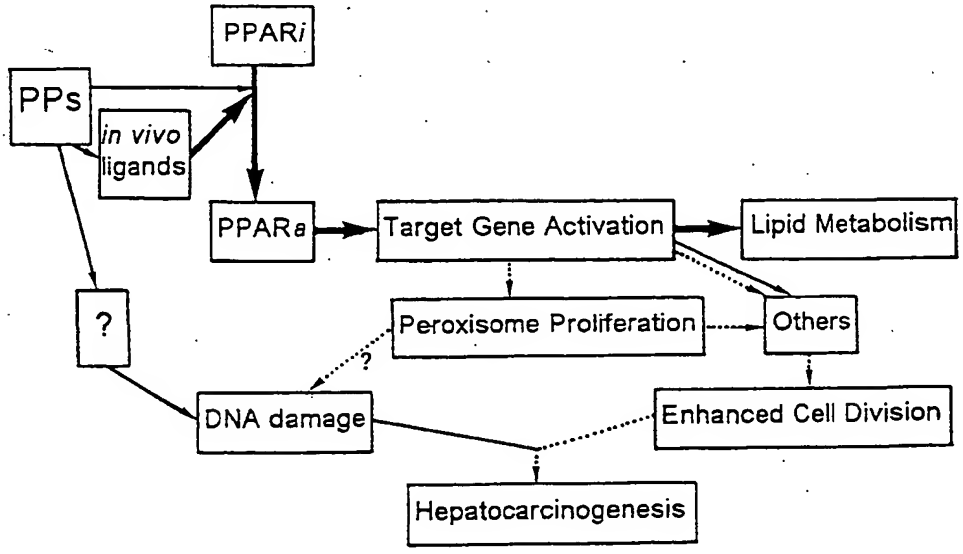


Fig. 5. Schematic diagram of PPAR-dependent and independent actions of PPs. Solid lines indicate essentially universal pathways and broken lines species-specific pathways.

ences in the responsiveness to PP among three isoforms (13), as well as those in the levels of expression of the three during development and in various tissues (13, 14) have been reported. Renaming of PPAR as fatty acid activatable receptor was also proposed (68).

The mechanism of the activation of PPAR by fatty acids is not known at present. The observation that fatty acids with various chain lengths and structures are active may be associated with the indirect mechanism: common intermediates or molecules of limited numbers are ultimate ligands of PPAR (21). However, fatty acids themselves may be the ligands of PPAR although its ligand specificity seems to be very low. Not all members of the steroid/thyroid/vitamin superfamily would have such high affinities and specificities as true hormone receptors. As suggested by O'Malley and Connelly (68), many of the orphan receptors in the superfamily would interact with environmental nutrients or metabolic intermediates with lower affinity and specificity. Their ligand binding sites may be similar to the substrate binding sites of metabolic enzymes, and consequently it would be difficult to detect the interactions. In addition, post-translational modification such as phosphorylation may be involved in activation of PPAR and this is not mutually exclusive with the ligand-directed mechanisms.

Specificity and diversity of PP action.

Species- and tissue-specific action is characteristic of PPs and the presence of PPAR must be involved in these specificities (23). Sex differences in the effects of PPs in one species are also evident and the activation of PPAR must be further modulated in a sex-dependent manner. The mechanistic analyses so far carried out have been limited to the core of transcriptional activation of the genes in lipid metabolism by PPAR, and only the characteristics of their specificity have been focused on to date. The extensive deletion analysis of the rat acyl CoA oxidase gene promoter by Osumi *et al.* (69) suggested the existence of a few DNA elements other than PPRE functioning positively or negatively. In addition to a counterpart of the heterodimer of the PPAR, many other protein factors will bind to the complex and the upstream elements of the PP-responsive promoter in a species- and tissue-specific manner, exerting specific expression of the gene depending on PPs. Extensive studies of other *cis*- and *trans*-acting factors are necessary and the issue of transcriptional regulation by PPAR has thus become one problem which involves a member of the steroid/thyroid/vitamin superfamily.

Most studies on the effects of PPs previously conducted have been focused on the specific and stimulatory action, primarily on peroxisomal gene expression or enzyme activities. However, analyses of other effects, which are not necessarily the result of peroxisome proliferation, on tissue-specific or fundamental cellular processes are also important for understanding the entire PP action including hepatocarcinogenesis. Recent studies have shown wide actions of PPs, such as down-regulation of transthyretin (63), apoAI and apoAIV (85), apoE (Motojima, K. and Goto, S., in preparation) and BiP/GRP78 (61) gene expression, and induction of elongation factor 2 (64) and one type of tropomyosin (Motojima, K., *et al.*, unpublished). At least some of them could be explained by the diverse function of PPAR generated by heterodimerization.

In addition to the possible existence of PPAR isoforms, the action of a PP can be further diversified by the PPAR's property to form heterodimers with other nuclear receptors. It has been shown that interaction of several nuclear receptors and factors with a specific DNA element is shown to depend on the orientation and spacing of half sites (17, 88). These characteristics will diversify the receptor-mediated transcriptional regulation. Transcriptional interference caused by heterodimerization of two nuclear receptors, such as RXR and RAR, has been shown to produce complex regulation (37, 50, 66, 67, 95), synergetic activation or severe inhibition of expression of the gene containing the same response element except for only a 1 bp difference (52). In addition to PPRE and RXRE, Kliewer *et al.* (40) showed by *in vitro* studies that the complex of PPAR and RXRα also strongly bound to a hormone-response element found in chicken ovalbumin (COUP-TF, which recognizes DR-1 (38, 79). COUP-TF was recently shown to bind PPRE and antagonize PP-mediated transcriptional activation (58a). This result suggests the possibility that the binding of the activated PPAR to PPRE can be modulated by several nuclear factors in the cell.). Thus it is plausible that the heterodimer complex also can bind to other untested 1 bp-spaced *cis* elements (DR-1) such as those recognized by ARP-1 (apoAI regulatory protein-1) (46) and HNF-4 (hepatocyte nuclear factor-4) (84). Heterodimerization of PPAR and ARP-1 might be involved in recently reported down-regulation of apoAI gene expression by fibrates (see above). Especially, the possibility of involvement of HNF-4 and/or its response element in PPAR-dependent transcriptional activation or inhibition is interesting because of their contribution in liver-specific gene expression. Furthermore, *in vitro* studies showed that PPAR-RXRα complexes also bound significantly to DR-5 (TRE in Moloney leukemia virus long-terminal repeat) and weakly to DR-4 element (RARE in RAR-β promoter) (40). Thus the target DNA sequences of the PPAR heterodimer complex may not be restricted to 1 bp-spaced element, suggesting further broadening and diversification of the responses mediated by PPAR. It was also reported that heterodimer formation sometimes causes a change in sequence specificity of the tar-

get (55). To date, there are no *in vivo* studies to indicate the diversified functions of PPAR by heterodimerization, and this kind of study would be difficult without information on the ligands for the orphan receptors. In this context, *in vivo* studies to show induction of peroxisomal β-oxidation genes in cultured hepatocytes by retinoic acid (25, 26) and effects of thyroid or steroid state in rats on the action of PPs (70, 73) are of interest.

PP-induced hepatocarcinogenesis.

Species- and tissue-specific carcinogenic properties of some PPs are unique and the mechanism of PP-induced hepatocarcinogenesis would include diverse functions of PPAR (22). Carcinogenesis involves multistage processes including tumor initiation triggered by DNA damage in cellular proto-oncogenes and growth-suppressor genes and tumor promotion by clonal expansion of the initiated cells. A PP may play multiple roles in carcinogenesis by acting with or without the aid of PPAR in various steps (see Fig. 5), and this model is in contrast to previous one-site acting models (see below).

Chronic administration of PP often causes development of hepatocellular carcinomas in rats and mice. Wy-14,643, a potent PP at 0.1% in the diet for 60 weeks, for example, resulted in a 100% incidence of rats with liver tumors (45). Classical genotoxic assays such as the Ames test have shown that PPs are non-genotoxic carcinogens. As the mechanism of liver carcinogenicity of PP, Reddy and Rao (74, 76) suggested the oxidative stress hypothesis that emphasized an imbalance in the induced levels of H_2O_2-producing peroxisomal oxidases and decomposing catalase activities. PP is regarded as a tumor initiator in this hypothesis. Cattley and co-workers (8, 56), on the contrary, suggested that PP may cause promotion of spontaneously initiated response rather than initiating the hepatocarcinogenic response in rodents. Despite much research, controversy remains as to whether PP-induced hepatocarcinogenicity is due to one of these causes, to both of them or to other causes, and the basic mechanism of the (probable) multistage carcinogenesis is unknown (60).

It is clear that there is no definitive association between peroxisome proliferation and hepatocarcinogenesis. Bezafibrate was reported as a potent PP but not carcinogenic (16, 24), and DEHP is known to produce far fewer hepatocarcinomas than Wy-14,643 does at doses causing comparable peroxisome proliferation (41, 45, 56). Thus peroxisome proliferation alone is not sufficient for carcinogenesis. But this does not exclude the possibility that PPs have initiating activity. Oxidative damage or other causes induced by peroxisome proliferation may play a role in the initiation step. A small increase in DNA adducts in rat liver by long-term exposure to a PP was reported (34). Furthermore, some PPs may exert initiating activity independently from peroxisome proliferation although most previous experiments for detecting initiating activity of PPs were negative (9, 18, 94). Recent studies measuring sister chromatid exchange, micronuclei formation and chromosomal aberrations as the genotoxic endpoints (28), in contrast to the Ames test, showed that several PPs including clofibrate, DEHP, Wy-14,643, nafenopin and ciprofibrate produced weak but significant genotoxic effects in primarily cultured hepatocytes (28, 78) and in hamster embryo cells (86) within a few hours, suggesting their actions were without transcriptional activation. The increase of replicative DNA synthesis in isolated hepatocytes within a few hours after administration of PPs to rats was also reported (89, 90). It is noteworthy that no clear relationship among induction of peroxisome proliferation, carcinogenicity *in vivo*, and genotoxicity in cultured cells has been observed. Furthermore, genotoxicity of PPs was also detected in human hepatocytes (28) where no peroxisome proliferation was observed. These results are inconsistent with a model of a single action in the initiation step even after the genotoxicity of PPs is established. Species- and tissue-specific carcinogenic properties of PPs do not seem to related to their genotoxicity.

PPs also may act as tumor promoters by modulating the expression of the genes involved in growth and differentiation. It is well known that PPs cause hepatic cell hyperplasia in a species-specific manner. The major objection to their having an important role in proliferative activities would be that a greatly decreased hyperplasia occurring after a transient increase only in the early phase of administration of PP cannot account for their critical role in carcinogenesis (74). Popp and colleagues (10, 56, 92) and another group (4), however, reported the increased proliferative activities of the carcinogenic PPs even in chronically PP-administered animals. Wy-14,643 actually promoted carcinogenesis in diethylnitrosamine (DEN)-initiated rat livers (10). Moreover, it would not be general hyperplasia but an activity to stimulate the proliferation of the initiated cells that plays a key role in carcinogenesis.

The target genes to alter the pathways allowing for the selective growth of the initiated cells in PP-induced hepatocarcinogenesis have not been identified. Various changes in many pathways create the potential to allow enhanced proliferation of and also inhibition of apoptosis of the initiated cells (82), but the number of studies to find such PP-induced changes is limited. Bieri (7) reported that *fos*, Ha-*ras*, and *raf* were activated by PPs and the levels of the mRNAs remained high as long as the PP treatment was prolonged. Their participation in tumorigenesis has not yet been proven.

The signal transduction system plays a central role in controlling cell growth and differentiation, and perturbation of the system would have significant effects on

cellular proliferation and thereby on carcinogenesis (81, 93). In addition to activation of *ras* (see above) which would disturb the signal transduction pathways, various changes in the patterns of *in vitro* phosphorylation of endogenous proteins have been detected (64), although their connection with cell growth has not yet been demonstrated. Histidyl phosphorylation of a membrane-associated protein having a molecular weight of 36 kDa (P36) was most evident among those changes; the P36 phosphorylation activity was induced in rat liver by the administration of PPs and activated *in vitro* by Ras protein and GTP, suggesting its involvement in a signal transduction pathway (62, 62a). Characterization of these changes and search for many others in various pathways will help in the identification of the key changes that lead to PP-induced carcinogenesis.

Modulation of gene expression would depend on diverse functions of PPAR caused by the complex formation with other nuclear receptors and transcriptional factors. Formation of such a transcriptional complex should provide a potential mechanism for species- and tissue-specific carcinogenic properties of PPs. However, quantitative relationships between cellular proliferation and carcinogenic responses have not been demonstrated, as reviewed by Melnick (57), and enhanced cell proliferation alone cannot be the primary mechanism by which PPs cause liver cancer.

None of these properties of PPs alone can quantitatively explain their species- and tissue-specific carcinogenicity. It seems to be important to consider the possibility that many PPs have some degree of both properties and their coupled actions effectively or ineffectively cause hepatocarcinoma. Interestingly, recent studies suggest that the cells under proliferation are much more sensitive to the exposure of very low doses of general genotoxic carcinogens (11, 33). Several PPs may be too weak in genotoxicity to give a positive detection, yet their associated species-specific function mediated by PPAR to enhance cellular proliferation of the initiated cells would make those PPs species-specific carcinogens.

The peroxisome has occasionally been put in the spotlight by the discovery of the presence of a β-oxidation system in it, the carcinogenic properties of PPs, and the discovery of several human disorders caused by the lack of it. However, the organelle has not claimed any general interest. The cloning of PPAR has again gained the attention of scientists, and the field of study of peroxisomes may expand this time because of the anticipated general importance of PPAR in lipid metabolism and its participation in diverse cellular processes. Further studies on structure and function of PPAR are necessary to elucidate the mechanisms of action of PPs, and these will provide invaluable information on biological regulation and be useful for drug designs, although all of the effects of PPs are probably not receptor-mediated.

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